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Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague–Dawley rats

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ABSTRACT

In order to assess the potential chronic toxicity and tumorigenicity of ammonium perfluorooctanoate (APFO), a 2-year dietary study was conducted with male and female rats fed 30 ppm or 300 ppm (approximately 1.5 and 15 mg/kg). In males fed 300 ppm, mean body weights were lower across most of the test period and survival in these rats was greater than that seen either in the 30 ppm or the control group. Non-neoplastic effects were observed in liver in rats fed 300 ppm and included elevated liver weight, an increase in the incidence of diffuse hepatocellular hypertrophy, portal mononuclear cell infiltration, and mild hepatocellular vacuolation without an increase in hepatocellular necrosis. Mean serum activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were elevated up to three times the control means, primarily at the 300 ppm dose. A significant increase in Leydig cell tumors of the testes was seen in the males fed 300 ppm, and tumors of the liver and acinar pancreas, which are often observed in rats from chronic exposure to peroxisome proliferating agents, were not observed in this study. All other tumor types were those seen spontaneously in rats of this stock and age and were not associated with feeding of APFO.

1. Introduction

The ammonium salt of perfluorooctanoic acid (APFO, CASRN 3825-26-1) has been used commercially as a surface-active agent in the production of various fluoropolymers. The toxicology of this chemical has been reviewed (Kennedy et al., 2004; Lau et al., 2007) covering both short and longer-term exposure studies as well as the standard toxicological endpoint studies. One of the key studies for hazard determination is a 2-year chronic toxicity and carcinogenicity bioassay in rats which has, to this point, only been available as a four-volume report on the United States Environmental Protection Agency public docket, Administrative Record AR-226. The study was conducted from April 1981 through May 1983 by the Pathology and Toxicology Department at Riker Laboratories, then a subsidiary of the 3M Company, St. Paul, MN. After this study was initially reported, Biegel et al. (2001) reported on a 2-year dietary bioassay of APFO in male rats which was conducted at a single dietary dose (300 ppm) equivalent to the high dose of the study reported herein. The Biegel et al. study was designed to further investigate the mode of action of APFO with emphasis on changes in response over a chronic dosing period.

Although the 3M study is an older study, the increased attention given to the potential health hazards of APFO in the scientific literature in recent years has prompted this detailed summary of the study to make the key findings and conclusions of the study more accessible. The study materials were audited in recent years and found to be complete and available. In addition, representative tissues from the study have been subjected to pathology peer review. These have included the pancreas (Frame and McConnell, 2003), the uterus and ovaries (Mann and Frame, 2004), and female mammary tissues (Hardisty et al., 2010).

2. Materials and methods

2.1. Test material

Ammonium perfluorooctanoate (APFO, FC-143, Lot 37, 97.2% pure (total of linear and branched isomers)) was supplied by 3M Company, St. Paul, MN. The APFO sample was determined to be stable over the course of the study based on analysis prior to the start of the study, approximately 1-year later, and at the termination of the exposure period.

2.2. Laboratory animals and husbandry

Three-hundred and sixty Sprague–Dawley rats (Crl:COBS@ CD(SD)BR, Charles River Company, Portage, MI) were obtained and quarantined for 10–14 days during which their suitability for testing was determined. At 39–41 days of age, three groups were formed using a table of random numbers and checked so that the starting group

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body weights were similar. The control and high dose groups contained 65 male and 65 female rats while the low dose group contained 50 rats of each sex.

Rats were housed in hanging stainless steel cages with wire mesh floors and fronts. The males were housed individually and the females two per cage. The control rats were housed in separate rooms from those receiving APFO in order to minimize possible cross contamination by potential vaporization and/or sublimation of the test article, which has a finite vapor pressure at room temperature. Air samples were taken from each of the animal housing rooms four months after the start of the test in order to assay for the presence of airborne chemical. Samples were found to contain below detectable levels of the fluorochemical. In addition to air monitoring, 30 untreated sentinel rats were placed in each of the two animal rooms. From each room, five male and five female sentinel rats were euthanized during the first week and at 1 and 3 months after the start of the study. Plasma samples from these rats was analyzed and found to contain less than 1 ppm organic fluorine.

Each animal room was temperature and humidity controlled with a 12/12 light/dark cycle. Individual rats were uniquely identified by an ear tag along with a cage card. Certified Purina Laboratory Chow (Ralston-Purina Co., St. Louis, MO) and tap water were provided ad libitum.

2.3. Diet preparation and analysis

APFO is a white powder which was added (in a stratified manner) directly into an appropriate quantity of untreated diet and mixed in a Hobart® blender (Hobart Company, Hobart, IN) for approximately 20 min for each separate batch. Prior to administering the diet to the rats, the test substance in the diet mixture was assayed. APFO was found to be uniformly blended and was tested and found to be stable in the ground feed.

Test article/diet mixtures were prepared fresh weekly during the study and representative samples of each were assayed for APFO content and homogeneity during the first month of the study and at 3 month intervals thereafter. The results of these assays indicated that the level of APFO was within a few percent of that desired.

2.4. Experimental design

The study consisted of three groups, one control (0 ppm APFO) and two treatment groups given either 30 ppm (low) or 300 ppm (high) APFO. An interim sacrifice at 1 year involved 15 male and 15 female rats from both the control and high dose groups. The remaining 50 rats per group continued on the study for the second year.

All rats were observed daily throughout the 2-year feeding period for signs of toxicity and mortality. Weekly physical examinations included palpation for the presence of masses. During the study, rats were closely monitored and euthanized when death appeared to be imminent in order to harvest non-autolyzed tissue for subsequent histopathological examination.

Body weights and feed consumption were recorded once per week for the first 6 months then once every 2 weeks for the remainder of the study. Eye examinations using indirect ophthalmoscopy and/or slit lamp biomicroscopy were performed on the control and high dose group by the staff veterinarian prior to the start of the test and at the 1-year interval. The eyes of the surviving control and high dose group rats were again examined by a consulting veterinary ophthalmologist 2–3 weeks prior to the end of the study at 2 years.

Clinical pathology determinations included hematology, clinical chemistry, and urinalysis. Tests were conducted on samples obtained from 15 rats per sex per group at 3, 6, 12, 18, and 24 months. Rats were randomly selected at each time interval.

Blood samples were collected from anesthetized rats which had been fasted overnight. Urine samples were obtained by placing each rat in an individual metabolism cage for 20–22 h.

Hematological tests included total red and white blood cell counts, hemoglobin, hematocrit, and a differential white cell count. Clinical chemistry parameters included total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, alkaline phosphatase (ALP), creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and calcium. Urine tests included pH, specific gravity, albumin, glucose, bilirubin, occult blood, and ketones.

At the 1 and 2 year sacrifices, samples of liver, blood, kidney, spleen, lung, and bone marrow (femur) were collected and frozen for possible future analysis.

Gross postmortem examinations were performed on all rats which died during the study and those which were terminated at the 1 year interim and 2 year necropsies. At necropsy, an examination was made of the external body surface and body orifices. The carcass was then opened and the contents of the abdomen, thorax, and cranium were examined *in situ* and following removal from the body.

Organ weights were obtained at the interim and terminal sacrifice rats. The weights of the adrenal glands, brain, testes, heart, kidneys, liver, spleen, and uterus were recorded for all interim sacrifice and 15 randomly selected final sacrifice rats/sex/group. Body weights were recorded just prior to sacrifice to use for calculation of organ to body weight ratios.

Representative samples of the following tissues and organs from each rat were fixed in 10% neutral buffered formalin for subsequent histologic processing: aorta, adrenals, brain (three sections including frontal cortex and basal ganglia, parietal cortex and thalamus, cerebellum and pons), eyes, gonads (ovaries, testes and epididymides), heart, small intestine (three sections), large intestine, kidney (two sections), liver (two sections), lung (two sections), mesenteric lymph node, mammary gland (female), pancreas, pituitary, salivary gland, spinal cord/bone marrow vertebrae), spleen, stomach, thyroid/parathyroid/trachea/esophagus, urinary bladder, uterus or prostate, any tissue masses (suspected tumors), and any gross lesions.

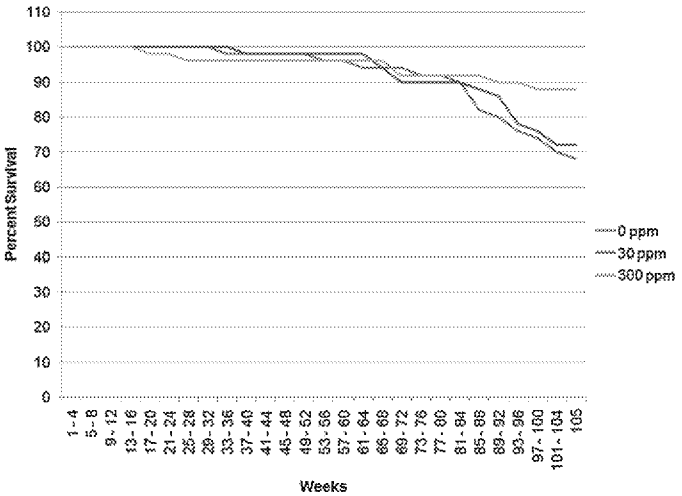

Fig. 1. Percent survival of male rats fed control diet or diet containing either 30 ppm or 300 ppm ammonium perfluorooctanoate for up to 2 years. Rats sacrificed at scheduled intervals were censored.

Light microscopic examination was performed on hematoxylin and eosin stained, paraffin-embedded tissue sections from all tissues listed above and from all rats in the control and high dose groups regardless of the cause of death. Microscopic examination of tissues from the low dose group included the tissues above except the aorta, brain, eyes, small and large intestine, lymph nodes, and spinal cord/bone marrow.

Statistical analysis: The means and standard deviations for body weights, feed consumption, absolute and relative (body and brain) organ weights and other laboratory data were determined separately for each sex and dose group. These data were analyzed using Bartlett's test for homogeneity of variance (Anderson and McLean, 1974). If this test was not significant at $\alpha = 0.001$, the data were further analyzed by comparing each treated group to the control group using a two-tailed Dunnett's test with $p \leq 0.05$ (Dunnett, 1964). If Bartlett's test was significant, the data were ranked (Conover and Iman, 1981) and a two-tailed Dunnett's test was performed on the ranks.

In addition, for each organ/lesion classification the sexes were analyzed separately using a two-tailed Fisher's Exact Test (Conover, 1971) comparing each treated group to the controls. An $\alpha = 0.05$ significance level with Bonferroni's adjustment for multiple comparisons was used within each organ/lesion/sex category. If the expected value of each cell was greater than 20, then Yates' corrected Chi-square (Fleiss, 1973) was used.

3. Results

3.1. Test article consumption

The APFO concentration measured as ppm in the diet was determined at 3 month intervals with a duplicate analysis performed when aberrant values were detected. The mean deviations from the target concentration of the low and high dose APFO groups were less than 3%. Actual APFO doses were determined for each 2 week period for each sex and each group and expressed as mg/kg per day. The mean test article consumption was 1.3 and 14.2 mg/kg for males and 1.6 and 16.1 mg/kg for females in the 30 and 300 ppm dietary dose groups, respectively.

3.2. Survival

Dietary treatment with APFO for 2 years did not adversely affect survival (Figs. 1 and 2). There were fewer deaths occurring in the 300 ppm dose group males and females than in the sex-matched controls. The final survival rates (based on 50 rats/sex/group at the end of the 104 weeks) in the control, 30 and 300 ppm dose groups,

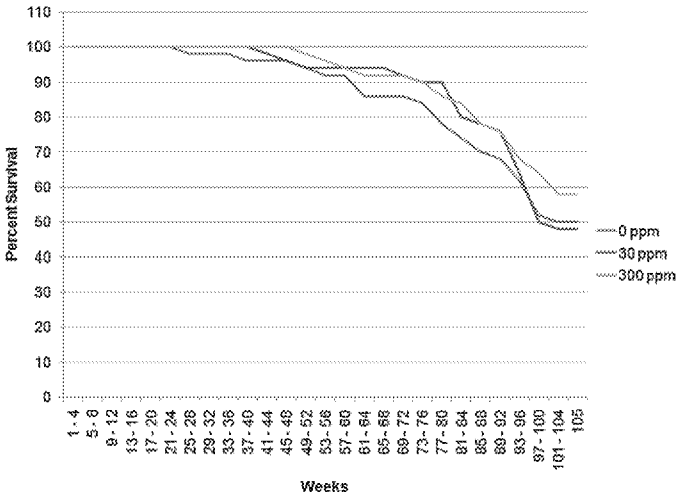

Fig. 2. Percent survival of female rats fed control diet or diet containing either 30 ppm or 300 ppm ammonium perfluorooctanoate for up to 2 years. Rats sacrificed at scheduled intervals were censored from analysis.

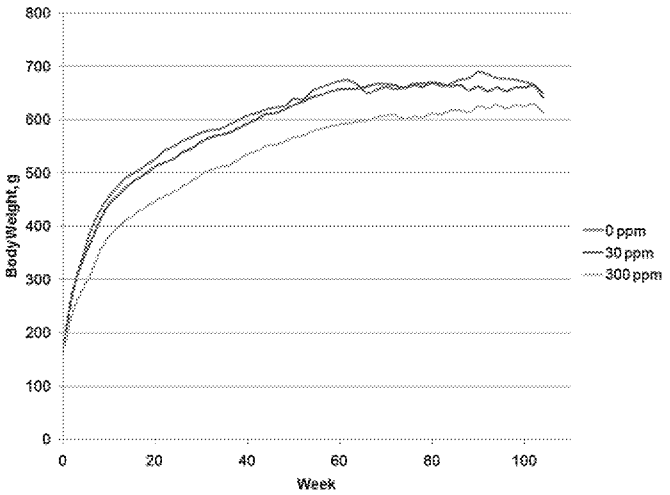

Fig. 3. Body weights of male rats fed control diet or diet containing either 30 ppm or 300 ppm ammonium perfluorooctanoate for up to 2 years.

respectively, were 70%, 72%, and 88% for males and 50%, 48%, and 58% for females. The increased survival rate observed in the high-dose male rats was statistically significant compared to the male controls.

3.3. Clinical signs

Clinical signs were those normally observed in rats of the same stock and age and were observed with equal frequency in both the APFO-treated and control rats. Ataxia was infrequently seen early in the test period with equal frequency in all groups and was more commonly associated with moribund animals. The incidence among females approaching termination of the study was higher in APFO-treated groups than in the control group with 2, 9, and 15 cases in the control, 30, and 300 ppm groups, respectively.

During the first and second months of the study, rats fed APFO experienced a suspected outbreak of sialodacryoadenitis (SDA) viral infection. Clinical signs included swollen submandibular salivary glands and occasional ocular manifestations. The submandibular swelling was resolved within 10 days and the incidence of ocular changes was extremely low. Similarly, control rats demonstrated signs of the condition during the sixteenth month of the study. Thirteen males and 13 females in the control group were involved, and the condition lasted approximately 16 days from the time of onset.

The incidence of palpable masses in the APFO-treated females was comparable to that of the control females. For males, there were more rats with masses in the controls (19, 7, and 10 in the control, 30, and 300 ppm dose groups, respectively), and when the numbers of palpable masses which regressed or resolved before the termination of the experimental period were evaluated, there remained fewer masses observed in the APFO-treated rats (11, 4, and 8, respectively).

3.4. Body weights

Body weight data for male and female rats are shown in Figs. 3 and 4, respectively. In male rats fed 300 ppm APFO, body weight gains were depressed in excess of 10% through 66 weeks of the study. The largest decrease was approximately 21% by week 6. This difference was statistically significant from week 2 of the study until week 98 when the control and 300 ppm male group weights gradually equalized. In the 30 ppm male group, a slight (5%) decrease in body weight was observed at week 6 with little additional decrease thereafter and all weights in this group were not statistically significantly different than those of the controls.

Mean body weights were not altered in female rats fed APFO through the first 18 months of the study. At 18 months, there was a gradual decrease in mean body weights in the 300 ppm group females which reached a maximum of 11% lower than the controls at 92 weeks.

3.5. Food consumption

In males, mean absolute food consumption was slightly decreased in the 300 ppm group for the first year of the study. Food consumption in the 30 ppm males, while somewhat inconsistent, was slightly increased during this same time period. Mean feed consumption relative to body weight was increased in all of the APFO-treated males throughout the study. This change was more pronounced in the 300 ppm group in which there was approximately a 13% increase noted with occasional values as high as 29% during the 2-year period. During the second year, the food consumption of both APFO-treated male groups was relatively stable and similar to that of the control group.

In the females, there was a trend, although somewhat inconsistent, toward lowered food consumption in both APFO-treated groups. The greatest decreases appeared to occur from 18 months

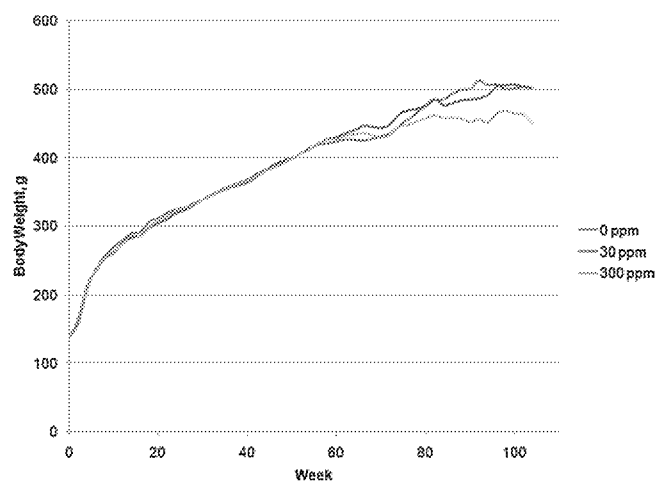

Fig. 4. Body weights of female rats fed control diet or diet containing either 30 ppm or 300 ppm ammonium perfluorooctanoate for up to 2 years.

to termination in both the 30 and 300 ppm groups. Overall, these variations appeared to be related to the observed variations in body weight among the female groups.

3.6. Hematology

Hematology data are shown in Table 1. In the 300 ppm male rats from 3 to 18 months, red blood cell counts, hemoglobin, and hematocrit values were minimally decreased. Statistically significant ($p < 0.05$) decreases were seen at various times in the erythrocytes, hemoglobin, and hematocrit. While some of these parameters were also altered in the 30 ppm males, the changes were of a lesser magnitude and, in some cases, were increased as well as decreased and appeared to be unrelated to treatment. Mean leukocyte counts were increased in both male groups through the first year of the study. These changes were due to increases in absolute counts of lymphocytes at 3 and 6 months and in neutrophils at 12 months.

No significant hematologic changes were seen in female rats fed APFO.

3.7. Clinical chemistry

Serum clinical chemistry results for male and female rats are presented in Tables 2 and 3, respectively. Among APFO-treated males from both groups, clinical chemistry findings at 3 months included slight increases in mean alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) and a moderate decrease in mean creatine phosphokinase (CPK). From 6 until 18 months, mean ALT, AST, and ALP values for the APFO-treated males were increased, sometimes with statistical significance, above the control values; whereas, at 24 months they appeared to be increased only in the 300 ppm group. Mean albumin concentrations were elevated with statistical significance in the 300 ppm male group relative to controls throughout the study and in the 30 ppm males at 3 and 6 months. In the APFO-treated males, activities of ALT, AST, ALP and albumin tended to decline over the period between 12 and 24 months, as did serum albumin in all male groups. Other than the changes noted above for males, there were occasional statistically significant differences between APFO-treated rats and their controls by sex, that were of small magnitude and inconsistent with time and dose. The latter were considered to be unrelated to APFO treatment.

3.8. Urinalysis and urine chemistry

Urinary findings in APFO-treated groups were consistent with those in control groups.

3.9. Ophthalmologic examinations

The results of the ophthalmoscopic examinations were consistent between APFO-treated and control rats. Changes that were observed included a random distribution of cataracts believed to be normal geriatric changes of the lens and some cases of chronic uveitis and superficial keratitis which were also considered to be within normal limits for aging populations of rats.

3.10. Pathology

3.10.1. One-year interim sacrifice

3.10.1.1. Gross pathology. Only the control and 300 ppm groups were examined during the 1-year interim sacrifice. The gross pathological findings observed at the 1-year interim sacrifice were unremarkable. A single 300 ppm group male had a small testes and 3/15 300 ppm group females had mammary masses compared to 1/15 in control group females.

3.10.1.2. Organ weights. Organ weight data (absolute and relative to body and brain weights) are shown in Table 4. There was a statistically significant increase in relative (to body weight) liver and kidney weights for APFO-treated males only. All 300 ppm APFO-treated male pituitary weight parameters were less than those of the controls. The male absolute and relative to brain adrenal weights were statistically significantly lower than those of controls; however, adrenal weights relative to body weights were not significantly different.

3.10.1.3. Microscopic pathology. Microscopic histopathological evaluation of the tissues from the rats necropsied at 1 year indicated that APFO-induced effects were confined to the liver. In male rats, diffuse hepatocellular hypertrophy (12/15 rats), focal hepatocellular necrosis (6/15), and portal mononuclear cell infiltration (13/15) were seen in the 300 ppm group, while the incidences in the control group were 0/15, 0/15, and 7/15, respectively. The principal compound-related liver change was hepatocellular hypertrophy which was characterized by an increase in size of liver parenchymal cells due to increased cytoplasmic volume. The increased cytoplasm was of a finely granular "ground glass" appearance. The coarser cytoplasmic organelles were relatively decreased and were displaced to the cell membrane. The nucleus:cytoplasm ratio was decreased by the increase in cytoplasm in affected cells. In affected livers, most or all lobules were involved and the centrilobular cells were more severely affected. Livers which had appreciable involvement with hepatocellular hypertrophy and cytoplasmic vacuolation also frequently had focal areas of necrosis; although, the incidence of necrosis was similar across all groups.

Findings other than those noted for liver included testicular tubular atrophy with marked aspermatogenesis which was observed in 2/15 300 ppm males but was not seen in the control males. The only change seen in the 300 ppm females was minimal to mild hepatocellular vacuolation with the incidence being 11/15 in the 300 ppm group compared to 5/15 in the control group.

3.10.2. Unscheduled and terminal sacrifices

3.10.2.1. Gross pathology. Gross findings seen in male 300 ppm rats undergoing unscheduled or terminal sacrifice included liver and testicular observations. The liver findings were a slight increase in the incidence of liver masses, nodules and raised lesions, mottled livers, and yellow or pale liver foci. While small testes were observed grossly in the control males as well as both treated groups, testicular masses were found in 1/50 30 ppm and 6/50 300 ppm rats with none seen in the controls. A very slight increase in mammary masses was seen in the 30 ppm females with masses observed in 27/50, 37/50, and 26/50 females in the control, 30, and 300 ppm groups, respectively. No remarkable gross liver changes were observed in the female rats. Other gross pathologic findings noted were those typical of findings in aging male and female Sprague–Dawley rats.

3.10.2.2. Organ weights. Organ weight data (absolute and relative to body and brain weights) are shown in Table 5. In both sexes, differences between groups in mean liver-weight parameters were not statistically significant. In both APFO-treated female groups, kidney weights as a percent of body weight were increased with statistical significance; however, the magnitude of change was minimal, there were no histological correlates, and kidney weights were not significant when normalized to brain weights. Male kidney weight parameters did not differ significantly from controls.

Table 1
Hematology parameters in male and female rats fed control diet or diet containing 30 ppm or 300 ppm ammonium perfluorooctanoate over a 24-month period. Values are group means ± SD with an *N* of 15 unless otherwise indicated.

Time (months) LNR[a]	Dose (ppm)	RBC (10^6 cells/mm^3) 5–8	HGB (g/dL) 11–17	HCT (%) 36–52	LEUK (10^3 cells/mm^3) 6–15	NEUT (10^3 cells/mm^3) 0.5–4.0	LYMP (10^3 cells/mm^3) 5–14
Males							
3	0	8.3 ± 0.3	16.4 ± 0.4	45.9 ± 1.0	12.7 ± 2.1	1.6 ± 0.6	10.7 ± 1.8
	30	7.9 ± 0.5	16.1 ± 0.7	**44.5** ± 1.9*	**15.2** ± 3.0*	2.1 ± 0.9	**12.8** ± 2.9*
	300	7.9 ± 0.7	15.5 ± 0.5	**43.3** ± 1.1*	14.9 ± 2.9	1.6 ± 0.9	**13.1** ± 2.2*
6	0	8.4 ± 0.4	15.1 ± 0.9	44.5 ± 2.7	10.7 ± 2.4	1.4 ± 0.7	8.8 ± 2.4
	30	**7.6** ± **0.3***	14.8 ± 0.8	43.9 ± 2.4	14.5 ± 3.2*	1.9 ± 1.1	**11.8** ± 3.0*
	300	**7.9** ± **0.4***	14.5 ± 1.0	43.1 ± 1.6	12.7 ± 2.2	2.0 ± 1.0	10.1 ± 2.3
12	0	8.4 ± 0.6[b]	15.9 ± 1.2[b]	46.7 ± 3.7[b]	7.9 ± 1.6[b]	1.1 ± 0.6[b]	6.4 ± 1.2[b]
	30	8.4 ± 0.4	15.5 ± 0.5	44.5 ± 1.9	**10.4** ± 1.7*	**2.2** ± 1.4*	7.6 ± 2.2
	300	**8.0** ± 0.5*	15.2 ± 0.7	**42.7** ± 2.0*	8.1 ± 1.8	**2.3** ± 0.6*	5.4 ± 1.2
18	0	7.5 ± 2.1	15.1 ± 3.1	40.8 ± 9.1	11.4 ± 7.3	5.1 ± 6.4	5.6 ± 1.5
	30	8.6 ± 1.1	15.8 ± 1.5	**46.1** ± 4.4*	11.2 ± 5.2	2.6 ± 3.3	**8.2** ± 2.6*
	300	**7.6** ± 0.6*	**14.8** ± 1.2*	**40.6** ± 3.0*	9.9 ± 1.5	2.8 ± 1.6	6.3 ± 1.1
24	0	7.8 ± 1.1	14.5 ± 1.6	42.3 ± 5.3	10.8 ± 7.5	3.7 ± 5.7	6.7 ± 2.1
	30	8.0 ± 0.9[b]	14.7 ± 1.2[b]	42.5 ± 4.2[b]	10.4 ± 3.5[b]	3.2 ± 2.0[b]	6.7 ± 2.2[b]
	300	7.9 ± 0.6	14.6 ± 1.4	43.3 ± 5.7	9.1 ± 2.4	2.4 ± 1.5	6.3 ± 1.6
Females							
3	0	6.9 ± 0.3	15.8 ± 0.5	43.7 ± 1.1	18.9 ± 2.7	2.6 ± 1.4	15.7 ± 3.0
	30	**7.3** ± 0.5*	16.0 ± 0.7	43.9 ± 1.8	18.0 ± 3.5	3.3 ± 1.3	14.2 ± 3.1
	300	6.7 ± 0.3	15.4 ± 0.6	**42.0** ± 18*	18.5 ± 3.8	3.2 ± 1.7	14.8 ± 2.8
6	0	7.3 ± 0.4	15.0 ± 0.6	42.3 ± 1.8	7.5 ± 2.5	1.4 ± 1.6	5.9 ± 2.2
	30	**6.9** ± 0.5*	**13.7** ± 1.0*	**38.7** ± 3.2*	7.2 ± 1.7	1.8 ± 1.0	5.1 ± 1.6
	300	7.4 ± 0.3	15.9 ± 0.6	42.5 ± 1.6	8.6 ± 2.0	1.6 ± 0.7	6.7 ± 1.8
2	0	7.6 ± 0.3	15.6 ± 0.4	44.3 ± 1.4	5.7 ± 2.1	1.3 ± 1.5	4.1 ± 1.0
	30	**7.3** ± 1.2*	**15.1** ± 1.7*	**42.5** ± 4.8*	5.8 ± 1.1	1.3 ± 0.7	4.1 ± 0.8
	300	6.9 ± 1.2	14.6 ± 1.7	40.5 ± 4.7	5.7 ± 1.8	1.4 ± 1.3	4.0 ± 1.1
18	0	7.4 ± 0.7	15.4 ± 1.4	42.9 ± 4.1	5.8 ± 2.2	1.0 ± 1.7	4.5 ± 1.0
	30	7.0 ± 0.8	14.8 ± 1.3	41.5 ± 3.8	6.8 ± 2.6	2.9 ± 2.2	3.5 ± 1.1
	300	7.2 ± 0.8	14.9 ± 1.3	41.9 ± 3.9	6.3 ± 2.4	2.2 ± 2.0	3.6 ± 1.3
24	0	7.1 ± 1.1	14.8 ± 1.8	42.3 ± 5.4	6.7 ± 4.1	2.9 ± 3.0	3.7 ± 1.3
	30	7.1 ± 1.1	14.6 ± 2.0	41.7 ± 6.0	8.6 ± 3.4	3.4 ± 1.0	4.0 ± 1.6
	300	7.1 ± 0.7	14.4 ± 1.3	40.4 ± 2.7	6.4 ± 1.7	2.2 ± 2.9	4.0 ± 1.2

Bolded values are statistically significant.
* Statistically significantly different than time- and sex-matched control mean (Dunnett's, $p \leq 0.05$).
[a] Laboratory normal range for conducting laboratory in the time frame of the study.
[b] $N = 14$.

Similarly, there were no changes in other organ–weight parameters that could be clearly associated with treatment.

3.10.2.3. Microscopic pathology – non-neoplastic. The incidences of relevant non-neoplastic microscopic findings for rats undergoing unscheduled or terminal sacrifice are presented in Table 6. The liver was the primary organ associated with non-neoplastic APFO-treatment related effects. Findings in rats sacrificed at unscheduled intervals or the terminal sacrifice were consistent with those observed in rats sacrificed during the 1-year interim sacrifice. Hepatocellular hypertrophy, cystoid degeneration, and portal mononuclear cell infiltration were the major dose-related changes seen in the liver of both sexes; however, hepatocellular necrosis was seen with essentially an equal distribution (incidences of 4–12%) across the three groups in both sexes. Hepatocellular hypertrophy was found at an incidence in males of 0%, 12%, and 80% in the control, 30, and 300 ppm groups, respectively; the corresponding female results were 0%, 2%, and 16%. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, was observed in male rats with an incidence of 8%, 14%, and 56%, for the control, 30, and 300 ppm male groups, respectively. The incidence in females was 2% in both treated groups and 0% in the controls. The incidence of hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, was slightly increased in the 300 ppm groups with incidence of 0%, 0%, and 6%, for the control, 30, and 300 ppm, respectively, in males with corresponding values for females of 2%, 0%, and 4%. The incidences of other hepatic changes such as basophilic hepatocyte alteration and/or chronic inflammatory changes consisting of portal mononuclear cell infiltration were slightly increased in the 300 ppm males but compared favorably to the relatively high incidence in the male controls.

The incidence of alveolar pulmonary macrophages and hemorrhage in 300 ppm male rats was increased relative to the control incidences; however, these changes were not believed to be related to treatment with APFO. This opinion was based on the observation that the incidence of chronic interstitial pneumonia and perivascular mononuclear infiltration was significantly reduced in these 300 ppm males, and pulmonary vascular mineralization was observed commonly in both control and APFO-treated rats.

Vascular mineralization of the testes occurred in 18% of the 300 ppm males with 0% and 6% seen in the controls and 30 ppm rats, respectively. The incidence of testicular tubular atrophy was only slightly increased with incidences of 14%, 20%, and 22% in the control, 30, and 300 ppm males, respectively, and these small increases are likely unrelated to treatment.

In females, an increase in tubular hyperplasia of the ovarian stroma was originally observed with incidences of 0%, 14%, and 32% in the control, 30, and 300 ppm groups, respectively. Tubular hyperplasia is considered to be a diffuse, non-neoplastic increase in the stromal tubular elements which is usually bilateral with decreased or absent follicular development and is common in aged rats. These tissues subsequently were subjected to peer review (Mann and Frame, 2004), and no statistically significant increases in ovarian stromal hyperplasia, adenoma, or adenoma and hyperplasia combined in treated groups relative to controls were found (Table 7).

Chronic sialadentitis, an inflammatory change of the salivary gland, often associated in rats with an antemortem viral infection, was increased in both the 30 and 300 ppm males. Hemosiderin, an iron-rich pigment, was found in greater concentrations in the

Table 2
Clinical chemistry values in male rats fed control diet or diets containing 30 ppm or 300 ppm ammonium perfluorooctanoate (APFO) over a 24-month period. Values are group means ± SD with an *N* of 15 unless otherwise indicated.

	Dose level (ppm)[a]	Length of dietary exposure				
		3 months	6 months	12 months	18 months	24 months
Glucose (mg/dL)	0	120.6 ± 13.74	119.0 ± 13.29	154.3 ± 26.17[b]	123.4 ± 26.39[b]	122.5 ± 23.07[b]
LNR[c] = 120–160	30	**134.6 ± 8.83**[*]	**134.9 ± 10.33**[*]	**131.1 ± 8.70**[#]	120.6 ± 27.62	121.3 ± 13.72[b]
	300	**129.5 ± 8.38**[*]	123.7 ± 11.17	138.7 ± 8.85	139.3 ± 16.14	**147.1 ± 13.26**[*]
BUN (mg/dL)	0	16.3 ± 1.84	17.5 ± 1.36	16.4 ± 2.50[b]	18.1 ± 3.38[b]	16.5 ± 3.16[b]
LNR = 10–30	30	17.1 ± 1.46	**15.9 ± 1.44**[*]	16.2 ± 1.15	**16.5 ± 6.48**[#]	16.6 ± 6.7[b]
	300	**19.4 ± 2.06**[*]	**20.7 ± 1.40**[*]	17.5 ± 1.73	17.6 ± 1.88	17.2 ± 5.03
ALT (IU/L)	0	21.4 ± 2.67	24.1 ± 3.75	33.5 ± 19.45[b]	34.1 ± 10.68[b]	33.4 ± 8.1[b]
LNR = 10–40	30	**34.5 ± 15.33**[#]	**53.3 ± 29.34**[#]	**77.6 ± 56.59**[#]	**59.7 ± 33.41**[#]	42.5 ± 10[b]
	300	**31.9 ± 21.94**[#]	**54.8 ± 29.26**[#]	**106.1 ± 70**[#]	**84.3 ± 55.95**[#]	**61.8 ± 20.13**[*]
AST (IU/L)	0	45.3 ± 7.26	49.7 ± 14.98	79.1 ± 44.61[b]	99.1 ± 68.14[b]	64.9 ± 25.76[b]
LNR = 20–60	30	59.7 ± 22.47	**92.1 ± 45.6**[#]	**124.4 ± 94.04**[#]	116.4 ± 57.99	68 ± 17.64[b]
	300	58.2 ± 27.23	**87.8 ± 34.83**[#]	**132.7 ± 76.84**[#]	123.3 ± 62.98	**95.7 ± 29.76**[*]
ALP (mg/dL)	0	91.1 ± 26.22	97.1 ± 40.41	105.8 ± 43.94[b]	85.2 ± 33.76[b]	70.1 ± 25.53[b]
LNR = 50–200	30	**138.7 ± 33.14**[*]	**146.9 ± 37.13**[*]	128.3 ± 41.75	112.5 ± 32.61	81.2 ± 26.2
	300	**153.5 ± 31.84**[*]	**147.3 ± 34.85**[*]	**166.5 ± 59.28**[*]	**184.4 ± 73.37**[*]	**113.5 ± 22.84**[*]
BILI (mg/dL)	0	0.6 ± 0.106	0.8 ± 0.19	0.7 ± 0.2[b]	0.9 ± 0.35[b]	0.4 ± 0.25[b]
LNR = 0.1–1.0	30	0.7 ± 0.09	**0.5 ± 0.14**[*]	0.8 ± 0.22	0.7 ± 0.25	0.4 ± 0.12
	300	0.8 ± 0.40	0.7 ± 0.22	0.6 ± 0.12	0.7 ± 0.31	**0.2 ± 0.06**[#]
ALB (g/dL)	0	4.4 ± 0.34	4.6 ± 0.20	3.7 ± 0.20[b]	3.2 ± 0.38[b]	2.8 ± 0.23[b]
LNR = 3.5–5.5	30	**4.7 ± 0.37**[*]	**5.0 ± 0.40**[*]	3.9 ± 0.26	3.2 ± 0.29	2.8 ± 0.30
	300	**5.1 ± 0.29**[*]	**5.4 ± 0.31**[*]	**4.4 ± 0.34**[*]	**3.7 ± 0.34**	**3.1 ± 0.27**[*]
TP (g/dL)	0	6.8 ± 0.34	7.0 ± 0.36	6.8 ± 0.27[b]	7.1 ± 0.61[b]	6.9 ± 0.44[b]
LNR = 5.5–7.5	30	6.6 ± 0.34	**6.7 ± 0.33**[*]	6.9 ± 0.33	7.2 ± 0.45	6.8 ± 0.39
	300	6.5 ± 0.31	**6.6 ± 0.28**[*]	6.8 ± 0.31	6.9 ± 0.42	6.9 ± 0.50
CPK (IU/L)	0	100.7 ± 26.46	89.9 ± 41.32	79.7 ± 47.61[b]	79.9 ± 74.52[b]	111.1 ± 68.04[b]
LNR = 50–150	30	**60.5 ± 12.65**[*]	**58.3 ± 31.23**[*]	67.6 ± 19.45	106.5 ± 69.23	**71.1 ± 20.55**[#]
	300	**67.0 ± 24.24**[*]	81.4 ± 26.66	68.3 ± 23.65	82.5 ± 45.87	88.3 ± 37.33
Ca^{2+} (mg/dL)	0	11.1 ± 0.68	11.5 ± 0.4	10.5 ± 0.3[b]	11.1 ± 0.93[b]	10.9 ± 0.43[b]
LNR = 10.2–12.5	30	11.0 ± 0.78	**10.9 ± 0.33**[*]	10.6 ± 0.29	10.4 ± 0.8[b]	**10.5 ± 0.31**[*]
	300	10.8 ± 0.5	**11.1 ± 0.22**[*]	10.6 ± 0.24	10.8 ± 1.1	10.8 ± 0.25

Bolded values are statistically significant.
[*] Statistically significantly different than the control value ($p \leq 0.05$) by Dunnett's *t*-test.
[#] Statistically significantly different than the control value ($p \leq 0.05$) by Dunnett's t-test on ranked data.
[a] Concentration of ammonium perfluorooctanoate in diet, w/w.
[b] $N = 14$.
[c] Laboratory normal range for conducting laboratory in the time frame of the study.

spleens of 300 ppm males and females but was seen in greatly reduced amounts in the 30 ppm males and females when compared to the controls. Other non-neoplastic lesions observed were those commonly associated with either endemic disease and/or geriatric changes found in this stock of rat.

3.10.2.4. Microscopic pathology – neoplastic. Neoplastic lesions observed in rats sacrificed at unscheduled intervals or terminal sacrifices are presented in Table 8. The only neoplastic lesion which could be related to APFO was an increase in testicular Leydig cell adenomas which was statistically significant in the 300 ppm group with an incidence of 0%, 4%, and 14% in the controls, 30, and 300 ppm groups, respectively.

Liver tumors were not increased with statistical significance in APFO-treated rats. There were no hepatocellular adenomas in male or female rats. The incidences of hepatocellular carcinomas were 6%, 2%, and 10% in the control, 30, and 300 ppm male groups, respectively; the corresponding female incidences were 0%, 0%, and 2%.

The incidence of female mammary gland adenoma was 15%, 31%, and 11% in the control, 30, and 300 ppm groups, respectively. For fibroadenomas, the incidences were 22%, 42%, and 48% in the control, 30, and 300 ppm groups, respectively. Mammary tissues from female rats were subjected to a pathology peer review using updated toxicological pathology criteria (Hardisty et al., 2010). The results of this effort resulted in a different distribution of tumor incidences. Both the original and peer-review results are presented in Table 8 and discussed below.

4. Discussion

This article covers the experimental details and results of a 2-year chronic toxicity and oncogenicity study of APFO in the rat. To date, only the laboratory report and subsequent pathology peer-reviews have been available since the study's completion in 1983. All of the study materials and records were audited and found to be available and reliable prior to conducting additional pathology reviews on mammary glands, ovaries, and pancreas. Presented in this article are the original findings as well as the results of subsequent pathology peer reviews.

The rats tolerated the 30 and 300 ppm APFO feeding levels which resulted in respective average daily intakes of 1.3 and 14.2 mg/kg for males and 1.6 and 16.1 mg/kg for females. Survival to term was excellent, with male rats of the 300 ppm group having statistically significantly increased survival as compared to controls. These same males weighed less throughout the study. This improved survival rate may be a reflection of the lower body weight. Keenan et al. (1999) studied the effect of moderate dietary restriction in rats and concluded that by limiting the daily intake of food, both lower body weights and lengthened

Table 3
Clinical chemistry values in female rats fed control diet or diets containing 30 ppm or 300 ppm APFO over a 24-month period. Values are group means ± SD with an *N* of 15.

	Dose level (ppm)[a]	Length of dietary exposure				
		3 months	6 months	12 months	18 months	24 months
Glucose (mg/dL)	0	138.9 ± 9.79	154.8 ± 14.98	134.9 ± 10.51	123.5 ± 15.51	122.2 ± 13.75
LNR[b] = 120–160	30	132.1 ± 12.06	**128.6 ± 13.41***	135.4 ± 10.35	122.2 ± 15.39	119.4 ± 15.23
	300	132.9 ± 10.96	**133.6 ± 9.61***	135.3 ± 8.18	120 ± 9.39	115.3 ± 18.72
BUN (mg/dL)	0	19.7 ± 1.62	21.6 ± 3.22	19.8 ± 2.14	18.1 ± 2.49	17.9 ± 4.1
LNR = 10–30	30	18.5 ± 2.07	19.1 ± 2	19.2 ± 2.83	20.1 ± 4.02	15.3 ± 3.35
	300	**24.5 ± 3.91***	20.7 ± 2.96	21.2 ± 5.21	18.7 ± 3.37	16.2 ± 3.76
ALT (IU/L)	0	22.6 ± 4.87	33.5 ± 17.2	34.1 ± 15.17	48.7 ± 11.6	40 ± 10.11
LNR = 10–40	30	19.4 ± 3.18	35.7 ± 14.55	37.1 ± 12.94	39.4 ± 14.31	38.6 ± 9.61
	300	26.2 ± 6.72	34.3 ± 20.46	38.6 ± 21.59	46.4 ± 6.36	43.1 ± 25.27
AST (IU/L)	0	48 ± 9.20	69.7 ± 38.56	68.1 ± 38.77	70.1 ± 24.4	67.8 ± 30.03
LNR = 20–60	30	44.9 ± 5.79	59.9 ± 28.6	72.6 ± 36.65	63.5 ± 25.21	63.5 ± 12.95
	300	51.6 ± 13.42	59.2 ± 29.11	66.2 ± 30.37	63.5 ± 18.97	67.0 ± 35.26
ALP (IU/L)	0	83.7 ± 32.29	77.8 ± 30.58	76.5 ± 30.01	65.5 ± 32.1	68.7 ± 33.39
LNR = 50–200	30	69.9 ± 25.98	80.9 ± 25.76	89.4 ± 29.87	**39.7 ± 13.47***	56.7 ± 19.9
	300	89.1 ± 25.36	82.1 ± 22.06	91.1 ± 42.86	76.1 ± 28.36	63.1 ± 19.69
BILI (mg/dL)	0	0.6 ± 0.18	0.6 ± 0.14	0.8 ± 0.38	0.6 ± 0.26	0.6 ± 0.31
LNR = 0.1–1.0	30	0.6 ± 0.09	0.6 ± 0.1	0.8 ± 0.21	0.6 ± 0.24	0.5 ± 0.21
	300	**0.5 ± 0.11***	0.5 ± 0.09	0.6 ± 0.21	0.6 ± 0.23	0.4 ± 0.17
ALB (g/dL)	0	5.3 ± 0.45	5.9 ± 0.65	4.3 ± 0.26	3.9 ± 0.23	3.4 ± 0.21
LNR = 3.5–5.5	30	5.0 ± 0.41	5.5 ± 0.66	4.4 ± 0.26	3.8 ± 0.26	3.3 ± 0.38
	300	5.0 ± 0.52	5.6 ± 0.46	4.2 ± 0.32	3.9 ± 0.28	3.4 ± 0.28
TP (g/dL)	0	7.2 ± 0.41	7.4 ± 0.64	7.3 ± 0.52	7.9 ± 0.57	7.6 ± 0.69
LNR = 5.5–7.5	30	7.0 ± 0.32	7.3 ± 0.57	7.5 ± 0.46	8.3 ± 0.73	7.2 ± 0.75
	300	**6.9 ± 0.41***	7.4 ± 0.34	7.1 ± 0.46	7.8 ± 0.47	7.4 ± 0.56
CPK (IU/L)	0	93.9 ± 41.44	56.5 ± 14.79	54.6 ± 20.02	37.3 ± 9.81	68.4 ± 32.23
LNR = 50–150	30	86.7 ± 21.06	89.2 ± 45.03	78.7 ± 78.42	27.6 ± 19.18	71.3 ± 35.73
	300	87.7 ± 37.12	66.7 ± 21.17	41.3 ± 11.52	35.1 ± 9.64	67.7 ± 27.06
Ca^{2+} (mg/dL)	0	11.0 ± 0.55	11.7 ± 0.53	11.2 ± 0.6	11.4 ± 0.53	11.5 ± 0.64
LNR = 10.2–12.5	30	**10.5 ± 0.69***	11.8 ± 0.45	11.0 ± 0.43	**10.6 ± 1.01***	11.3 ± 0.45
	300	11.1 ± 0.52	11.7 ± 0.43	11.1 ± 0.33	11.1 ± 0.43	11.2 ± 0.46

Bolded values are statistically significant.
* Statistically significantly different than the control value ($p \leq 0.05$) by Dunnett's *t*-test.
[a] Concentration of ammonium perfluorooctanoate in diet, w/w.
[b] Laboratory normal range for conducting laboratory in the time frame of the study.

survival could be achieved. The cumulative adverse effects of *ad libitum* feeding (as used in this study) can manifest in cardiovascular disease, progressive decline of kidney function, multiple endocrine organ dysfunctions, early onset of diet-related tumors, and ultimately higher mortality. In this study, food consumption among 300 ppm males was lower during the first year of the study and was reflected in lower body weights. Females in the 300 ppm group weighed somewhat less than did the corresponding controls but only towards the end of the study, and survival rates in this female group were only marginally greater. No significant adverse clinical signs were observed in any APFO-treated group.

The incidence of one clinical sign, ataxia, had incidences in primarily moribund female rats of 3%, 18%, and 23%, at 0, 30, and 300 ppm APFO, respectively. The corresponding incidence in male rats was 6%, 10%, and 3%, respectively. The lack of a dose-related increase in ataxia in male rats, which pharmacokinetic studies have shown to have a higher body burden of PFOA (Kennedy et al., 2004), suggests that the subjective observations of ataxia in the females was secondary to their age and moribund state and not related to treatment. Ataxia was not reported in a second 2-year dietary bioassay of male rats (Biegel et al., 2001), two 90-day dietary studies in rats (Griffith and Long, 1980; Perkins et al., 2004), and in a two-generation reproduction study in rats (Butenhoff et al., 2004), all conducted at doses similar to those used in the study reported herein. Thus, ataxia does not appear to be a APFO-treatment-related finding.

With respect to hematology, there were occasional decreases in red cell parameters in male and female rats, being more frequent in males of the 300 ppm dietary group. These were not consistent within group over time and were mostly small in magnitude. Sporadic, non-dose-related increases in white cell elements were observed in male rats. Neither of these findings was suggestive of adverse effects on hematological parameters. Decreases in red cell parameters within the normal range are frequently associated with the high doses in toxicological studies and generally considered not to be of toxicological significance (Car et al., 2006).

The clinical chemistry finding in male rats fed APFO that were most consistent over time and showed some dose–response, with mean values occasionally ≥2 times the control and/or outside the laboratory normal range were for the liver-associated enzymes, ALT and AST. Mean plasma activities of ALT in 50% of the APFO-treated male samples were 2–3 times their time-matched control means, with the response at 300 ppm being somewhat greater than at 30 ppm. Of these, 80% were above the laboratory normal range. None of the mean plasma AST activities for APFO-treated males were ≥2 times their time-matched control means, and 8 of the APFO-treated means and three of the control means were above the laboratory normal range. For ALP, although 70% of the APFO-treated male mean plasma activities were statistically significantly greater than their time-matched controls, only in one instance (10%) was the mean value more than twice the time-matched control value, and none of the ALP activity measurements were greater than the laboratory normal range. The elevations of ALT, AST, and

Table 4
Interim organ weight data for male and female rats in either control groups or groups fed 300 ppm APFO in their diet for 1 year. Organ weights are given as absolute weight in grams, as a percent of body weight, and as ratio of organ weight to brain weight. Bolded values are statistically significant.

Organ	Dietary dose group (ppm APFO)			
	Males		Female	
	0	300	0	300
Body	625.5 ± 65.9	580.8 ± 51.2	401.1 ± 77.2	403.1 ± 66.7
Brain	2.11 ± 0.11	2.06 ± 0.07	1.96 ± 0.13	1.97 ± 0.12
(Ratio to Body)	0.34 ± 0.03	0.36 ± 0.03	0.51 ± 0.11	0.50 ± 0.08
Liver	19.15 ± 3.21	21.33 ± 2.50	12.28 ± 1.90	12.90 ± 1.78
(Ratio to Body)	3.05 ± 0.32	**3.70 ± 0.51***	3.09 ± 0.29	3.24 ± 0.42
(Ratio to Brain)	9.05 ± 1.32	**10.34 ± 1.18***	6.27 ± 1.00	6.55 ± 0.83
Heart	1.63 ± 0.16	**1.45 ± 0.05***	1.12 ± 0.13	1.09 ± 0.12
(Ratio to Body)	0.26 ± 0.02	0.25 ± 0.02	0.28 ± 0.04	0.27 ± 0.04
(Ratio to Brain)	0.77 ± 0.07	**0.71 ± 0.04***	0.57 ± 0.07	0.55 ± 0.07
Spleen	0.89 ± 0.14	0.80 ± 0.11	0.66 ± 0.12	0.61 ± 0.12
(Ratio to Body)	0.14 ± 0.03	0.14 ± 0.02	0.17 ± 0.03	0.16 ± 0.05
(Ratio to Brain)	0.42 ± 0.07	0.39 ± 0.06	0.34 ± 0.06	0.31 ± 0.06
Kidneys	3.85 ± 0.51	4.05 ± 0.42	2.53 ± 0.38	2.60 ± 0.28[a]
(Ratio to Body)	0.62 ± 0.06	**0.70 ± 0.09***	0.64 ± 0.09	0.65 ± 0.11[a]
(Ratio to Brain)	1.82 ± 0.19	1.96 ± 0.23	1.29 ± 0.20	1.32 ± 0.12[a]
Adrenals	0.068 ± 0.013	**0.055 ± 0.008***	0.076 ± 0.012	0.080 ± 0.019
(Ratio to Body)	0.011 ± 0.002	0.010 ± 0.001	0.020 ± 0.005	0.020 ± 0.006
(Ratio to Brain)	0.032 ± 0.006	**0.027 ± 0.004***	0.039 ± 0.006	0.041 ± 0.010
Testes	3.55 ± 0.42	3.50 ± 0.33	–	–
(Ratio to Body)	0.570 ± 0.057	0.598 ± 0.064	–	–
(Ratio to Brain)	1.68 ± 0.15	1.68 ± 0.13	–	–
Uterus	–	–	0.78 ± 0.25	0.71 ± 0.22
(Ratio to Body)	–	–	0.201 ± 0.077	0.181 ± 0.074
(Ratio to Brain)	–	–	0.40 ± 0.13	0.36 ± 0.11
Pituitary	0.021 ± 0.006	**0.012 ± 0.004***	0.023 ± 0.006	0.020 ± 0.007
(Ratio to Body)	0.003 ± 0.001	**0.002 ± 0.001***	0.006 ± 0.002	0.005 ± 0.002
(Ratio to Brain)	0.010 ± 0.003	**0.005 ± 0.002***	0.012 ± 0.004	0.010 ± 0.003

* $p \leq 0.05$, two tailed Dunnett's *t*-test on raw data.
[a] $N = 14$.

ALP observed in APFO-treated males may, in part, be due to the hepatic hypertrophic (inductive) effect of APFO and/or may also represent borderline chronic liver toxicity (Amacher, 1998; Boone et al., 2005).

All other mean clinical chemistry values in APFO-treated rats that reached statistical significance relative to their time-matched controls were within the laboratory normal range for the measured parameter. Mean serum albumin in 300 ppm APFO-treated males was elevated compared to the control values through the study; however the 30 ppm APFO-treated males did not show this effect. The increased serum albumin may have been related to the anti-inflammatory effect of PPARα activation (Gervois et al., 2004). Other than the changes noted above for males, there were occasional statistically significant differences between APFO-treated rats and their controls by sex, that were of small magnitude, inconsistent with time and dose, and were considered to be unrelated to APFO treatment.

Other than liver, organ weights appeared to be unaffected by treatment with APFO. A relatively small increase in relative liver weights was observed in males fed 300 ppm at the 1 year interim but not at the 2-year sacrifice. Increased male rat liver weights have been a consistent finding in toxicological studies of APFO. The magnitude of liver weight increases, both absolute and relative, observed in the 2-year study by Biegel et al. (2001) and the one-month study of Elcombe et al. (2010) at the same dietary level (300 ppm) were more pronounced. The liver-weight increase observed in the study here reported was consistent with clinical chemistry and microscopic findings.

Although there were statistically significant changes in the weight parameters for some other organs, these findings did not appear to be causally associated with APFO treatment in that they were of small magnitude, were not associated with histopathological correlates, were often not present when adjusted to body or brain weights, or did not show a dose–response. It should be noted that, just as with clinical chemistry determinations, organ weight data for rats at 2-years is of lesser importance and can be potentially misleading in the determination of treatment-related changes (Sellers et al., 2007).

Differences in pharmacokinetics between male and female rats have been well-established with female rats having a much higher capacity to eliminate PFOA in urine than males (Kudo and Kawashima, 2003). Thus, at a given dietary dose, area under the concentration-time curve (AUC) can be inferred to be less for the female rat. This difference in pharmacokinetics likely accounts for the lack of reduced responsiveness of APFO-treated female rats as compared to males.

In the study reported here, the liver enlargement was small in magnitude, and no increases in liver tumors were observed; whereas, in the Biegel et al. study, a more striking increase in liver weight was observed and the incidence of hepatocellular adenoma was increased. However, it should be noted that, in the study reported herein, the incidence of hepatic hyperplastic nodules was somewhat increased in the 300 ppm group. Diagnostic criteria for this finding, which represents a regenerative process, have changed since the study was originally evaluated (Maronpot et al., 1986). The association of hepatocyte vacuolation and focal necrosis with

Table 5
Organ weight data for male and female rats in either control groups or groups fed 30 or 300 ppm APFO in their diet for 2 years (N = 15/sex/group). Organ weights are given as absolute weight in grams, as a percent of body weight, and as ratio of organ weight to brain weight.

Organ	Dietary dose group (ppm APFO)					
	Males			Female		
	0	30	300	0	30	300
Body	666.7 ± 99.8	648.2 ± 107.0	632.6 ± 91.9	512.5 ± 82.6	533.3 ± 82.5	451.2 ± 68.1
Brain	2.11 ± 0.13	**2.20 ± 0.13***	2.19 ± 0.07	2.03 ± 0.10	2.03 ± 0.13	2.05 ± 0.11
(Ratio to Body)	0.32 ± 0.05	0.35 ± 0.06	0.35 ± 0.05	0.50 ± 0.08	0.38 ± 0.06	0.47 ± 0.08
Liver	18.15 ± 0.07	18.81 ± 3.43	19.12 ± 3.04	15.11 ± 3.75	17.06 ± 3.85	14.05 ± 2.74
(Ratio to Body)	2.73 ± 0.34	2.93 ± 0.53	3.04 ± 0.41	2.94 ± 0.47	3.20 ± 0.55	3.13 ± 0.42
(Ratio to Brain)	8.65 ± 1.59	8.54 ± 1.49	8.76 ± 1.42	7.46 ± 1.92	8.46 ± 2.16	6.88 ± 1.51
Heart	1.72 ± 0.23	1.81 ± 0.31	1.67 ± 0.30	1.37 ± 0.20	1.49 ± 0.19	1.31 ± 0.17
(Ratio to Body)	0.26 ± 0.04	0.28 ± 0.06	0.27 ± 0.05	0.27 ± 0.04	0.28 ± 0.05	0.30 ± 0.06
(Ratio to Brain)	0.82 ± 0.10	0.82 ± 0.14	0.76 ± 0.12	0.67 ± 0.10	0.74 ± 0.12	0.64 ± 0.10
Spleen	1.22 ± 0.34	1.11 ± 0.21	1.16 ± 0.31	0.80 ± 0.14	**1.15 ± 0.68#**	0.70 ± 0.20
(Ratio to Body)	0.19 ± 0.06	0.17 ± 0.04	0.18 ± 0.05	0.16 ± 0.03	**0.21 ± 0.08***	0.20 ± 0.05
(Ratio to Brain)	0.58 ± 0.16	0.50 ± 0.09	0.53 ± 0.15	0.39 ± 0.08	**0.57 ± 0.33#**	0.34 ± 0.10
Kidneys	4.48 ± 0.75	5.19 ± 1.78	4.59 ± 0.53	3.13 ± 1.29	**3.48 ± 0.45***	3.16 ± 0.47
(Ratio to Body)	0.69 ± 0.10	0.82 ± 0.35	0.74 ± 0.11	0.62 ± 0.08	0.66 ± 0.10	**0.71 ± 0.13***
(Ratio to Brain)	2.10 ± 0.98	2.36 ± 0.83	2.10 ± 0.23	1.54 ± 0.17	1.72 ± 0.23	1.55 ± 0.26
Adrenals	0.080 ± 0.018	0.090 ± 0.027	0.087 ± 0.018	0.103 ± 0.046[a]	0.137 ± 0.044	0.113 ± 0.039
(Ratio to Body)	0.012 ± 0.003	0.014 ± 0.005	0.014 ± 0.003	0.020 ± 0.008[a]	0.026 ± 0.007	0.025 ± 0.018
(Ratio to Brain)	0.038 ± 0.010	0.041 ± 0.013	0.040 ± 0.008	0.050 ± 0.022[a]	0.068 ± 0.023	0.055 ± 0.019
Testes	3.48 ± 0.70	3.70 ± 0.73	3.63 ± 0.64	–	–	–
(Ratio to Body)	0.54 ± ±0.14	0.57 ± 0.09	0.58 ± 0.10			
(Ratio to Brain)	1.66 ± 0.34	1.68 ± 0.30	1.66 ± 0.29			
Uterus	–	–	–	0.81 ± 0.29	0.86 ± 0.49	0.82 ± 0.38
(Ratio to Body)				0.16 ± 0.06	0.16 ± 0.09	0.18 ± 0.08
(Ratio to Brain)				0.40 ± 0.16	0.42 ± 0.23	0.40 ± 0.17

[a] N = 14.
* $p \leq 0.05$, two tailed Dunnett's t-test on raw data.
# $p \leq 0.05$, two tailed Dunnett's t-test on ranked data.

hepatocellular hypertrophy was more evident in rats at the scheduled 1-year sacrifice than in rats sacrificed between 1 and 2 years (because of the higher background incidence of these findings in older rats) and suggests that the progression of lesions could be from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia.

It has been demonstrated that the observed APFO-induced hepatomegaly is characterized by induction of the xenosensor nuclear receptors PPARα (NR1C1), CAR (NR1I3), and PXR (NR1I2) leading to an early hepatocellular proliferative response with resulting hepatocellular hypertrophy and hyperplasia of the liver (Elcombe et al., 2010). In rodents, hepatocellular proliferation and, possibly, decreased hepatocellular apoptosis, as a result of activation of the xenosensor nuclear receptors PPARα, CAR, and PXR, may predispose to liver tumor formation (Klaunig et al., 2003; Lake, 2009; Ross et al., 2010). Evidence exists to suggest that human hepatocytes are less sensitive to the inductive and proliferative effects resulting from PFOA-mediated induction of these nuclear receptors (Bjork et al., 2011; Bjork and Wallace, 2009; Nakamura et al., 2009). If cell proliferation is central to the mode of action for development of liver tumors from exposure to agents that activate these nuclear receptors, such as APFO, the putative inability of the human receptors to support the stimulation of the hepatocellular proliferation and hyperplasia would lessen the likelihood of a hepatocarcinogenic effect (Hirose et al., 2009; Ross et al., 2010; Yang et al., 2008). This conclusion is supported by human cancer mortality studies on therapeutically administered agents such as phenobarbital, and fibrate drugs, which are CAR/PXR and PPARα agonists, respectively (Bentley et al., 1993; Doull et al., 1999; IARC, 2001; Olsen et al., 1989; Olsen et al., 1995; Whysner et al., 1996). In addition, as discussed in detail below, epidemiological studies of PFOA-exposed human populations, including workers, have not found clinically-relevant associations with liver toxicity or mortality from liver cancer (Emmett et al., 2006; Eriksen et al., 2009; Leonard et al., 2008; Lundin et al., 2009; Olsen and Zobel, 2007).

In the original analysis of mammary tissues from female rats, the incidence of fibroadenoma of the mammary gland in the female 300 ppm group (48%) was greater than that in either the concurrent control group (22%) and similar to that of the 30 ppm group (42%) but was considered to be within the norm for background variation of this lesion in Sprague–Dawley rats based on normal background incidence from the published literature (see Table 5 of Hardisty et al., 2010). To confirm this, a Pathology Working Group (PWG) was commissioned to review the female mammary tumor findings, blinded to treatment status, using current diagnostic criteria (Hardisty et al., 2010). The principal difference between the original reported findings and the PWG results involved changes in the mammary gland that were initially reported as lobular hyperplasia, which the PWG felt had features more characteristic of mammary-gland fibroadenoma. As a result, the numbers of rats with benign tumors (adenoma and fibroadenoma) were reclassified from 13 to 19 in the control group, from 19 to 22 in the 30-ppm group, and from 21 to 23 in the 300 ppm group. Although the incidence of neoplasms varied among the control and treated groups, there were no statistically significant differences when evaluated using the Fisher's exact test for pairwise comparison for fibroadenoma, adenocarcinoma, total benign neoplasms, and total malignant neoplasms. The morphologic appearance, overall incidence, and distribution of the neoplasms observed in treated and control groups were similar.

The incidence of Leydig cell adenoma was increased with statistical significance in male rats fed 300 ppm APFO. The 14% incidence observed at 300 ppm in this study is similar to the incidence of 11%

Table 6
Incidence of relevant non-neoplastic microscopic findings for male and female rats in either control groups or groups fed 30 ppm or 300 ppm APFO in their diet for up to 2 years.

Organ/lesion	Dietary dose group (ppm APFO)					
	Males			Females		
	0	30	300	0	30	300
Adrenal						
Nodular hyperplasia	2/49 (4)[a]	1/50 (2)	9/50 (18)	0/50 (0)	3/50 (6)	1/49 (2)
Sinusoidal ectasis	11/49 (22)	13/50 (26)	16/50 (32)	42/50 (84)	43/50 (86)	41/49 (82)
Heart						
Myocarditis, chronic	14/48 (28)	18/45 (36)	17/47 (34)	16/50 (32)	**5/50 (10)***	10/49 (20)
Kidney						
Chronic progressive nephropathy	44/48 (92)	43/45 (96)	43/47 (91)	30/50 (60)	21/50 (42)	26/50 (52)
Liver						
Cystoid degeneration	4/50 (8)	7/50 (14)	**28/50 (56)***	0/50 (0)	1/50 (2)	1/50 (2)
Hepatocellular altered basophils	2/50 (4)	1/50 (2)	6/50 (12)	8/50 (16)	8/50 (16)	2/50 (4)
Hyperplastic nodule	0/50 (0)	0/50 (0)	3/50 (6)	1/50 (2)	0/50 (0)	2/50 (4)
Hepatocellular hypertrophy	0/50 (0)	6/50 (12)	**40/50 (80)***	0/50 (0)	1/50 (2)	**8/50 (16)***
Portal mononuclear cell infiltrate	37/50 (74)	32/50 (64)	**48/50 (96)***	19/50 (38)	11/50 (22)	19/50 (38)
Necrosis	3/50 (6)	5/50 (10)	5/50 (10)	5/50 (10)	6/50 (12)	2/50 (4)
Lung						
Alveolar macrophages	10/49 (20)	16/50 (32)	**31/49 (62)***	14/50 (28)	10/50 (20)	19/50 (38)
Hemorrhage	10/49 (20)	14/49 (28)	**22/50 (44)***	14/50 (28)	13/50 (26)	19/50 (38)
Perivascular mononuclear infiltrate	21/49 (42)	**3/49 (6)***	**7/50 (14)***	13/50 (26)	**2/50 (4)***	14/50 (28)
Vascular mineralization	43/49 (86)	43/49 (86)	47/50 (94)	22/50 (44)	**38/50 (76)***	26/50 (52)
Pneumonia, interstitial	16/49 (32)	**5/49 (10)***	7/50 (14)	7/50 (14)	3/50 (6)	9/50 (18)
Ovary						
Cyst	–	–	–	6/48 (12)	9/50 (18)	5/47 (11)
Tubular hyperplasia[b]	–	–	–	0/48 (0)	**7/50 (14)***	**15/47 (32)***
Pancreas						
Acinar atrophy	6/46 (13)	9/46 (20)	11/49 (22)	6/49 (12)	5/43 (12)	4/47 (9)
Hyperplasia, acinar cell	0/46 (0)	2/46 (4)	2/49 (4)	0/49 (0)	0/43 (0)	0/47 (0)
Hyperplasia, islet cell	2/46 (4)	2/46 (4)	4/49 (8)	0/49 (0)	1/43 (2)	1/47 (2)
Salivary gland						
Sialadentitis, chronic	1/44 (2)	**12/44 (27)***	**14/46 (30)***	1/43 (2)	1/46 (2)	2/40 (5)
Spleen						
Hemosiderosis	16/50 (32)	**4/50 (8)***	22/50 (44)	25/50 (50)	**3/47 (6)***	**12/50 (24)***
Testis/epididymis						
Tubular atrophy	7/49 (14)	10/49 (20)	11/50 (22)	–	–	–
Vascular mineralization	0/49 (0)	3/50 (6)	**9/50 (18)***	–	–	–
Thyroid						
Hyperplasia, C-cell	1/43 (2)	6/47 (13)	1/47 (2)	0/50 (0)	1/49 (2)	3/49 (6)
Hyperplasia, follicular cell	1/43 (2)	2/47 (4)	1/47 (2)	1/50 (2)	0/49 (0)	0/49 (0)
Uterus						
Cystic glands	–	–	–	7/50 (14)	12/49 (24)	5/48 (10)

Bolded values are statistically significant.
* Statistically significantly different from controls ($p \leq 0.05$).
[a] Number observed/number examined (%).
[b] See also Table 7 and Mann and Frame (2004) for pathology peer review of ovarian tissues.

reported by Biegel et al. (2001). In both this study and the Biegel et al. study, there were no Leydig cell adenomas observed in control rats; however, the incidence of Leydig cell adenoma in the 30 ppm group of this study (4%) was within expected background rates and not considered to be treatment-related. Butenhoff et al. (2004) calculated a benchmark dose for Leydig cell adenoma based on study data and determined that the lower 95% confidence limit of the benchmark dose for a 10% increase in incidence was 100 ppm. It has been suggested that APFO-induced Leydig cell tumors may result from hormonal changes brought about by induction of aromatase (Biegel et al., 1995; Liu et al., 1996a,b).

In contrast with the study of Biegel et al. (2001), in which the incidences of pancreatic acinar cell adenoma were 0/80, 1/79 (1.3%), and 7/76 (9.2%) in control, control pair-fed, and 300 ppm APFO groups, respectively, there were no pancreatic acinar cell adenomas reported in any group in this study. The lack of pancreatic acinar cell tumors in this study prompted a pathology review of male pancreatic tissues from this and the Biegel et al. study (Frame and McConnell, 2003). Typically, cytological features that clearly distinguish hyperplasia from adenoma are not readily apparent (Eustis and Boorman, 1990; Hansen et al., 1995), and terms such as hyperplasia and adenomatous hyperplasia to describe non-neoplastic acinar cell lesions have been used (Greaves and Faccini, 1992). To achieve some uniformity, diagnostic criteria were established to differentiate acinar cell hyperplasia from adenoma, with the criterion most commonly employed being the two-dimensional size of the lesion (Eustis and Boorman, 1990; Hansen et al., 1995), thus reflecting the difference in the area of a random section through the lesion rather than differences in biological potential. The pathology review found that exposure to 300 ppm APFO in this study was associated with a slight increase in acinar cell hyperplasia but not adenoma or carcinoma.

In the original analysis of ovarian tissues, an equivocal increase in the incidence of ovarian (stromal) tubular hyperplasia in both

Table 7
Results of pathology peer review of ovaries showing reevaluation of incidence of gonadal stromal hyperplasia and adenoma in female rats in either control group or groups fed 30 ppm or 300 ppm APFO in their diet for up to 2 years.[a]

Dose group (ppm APFO)	0	30	300
Number examined	45	47	46
Hyperplasia	8	16	15
Adenoma	4	0	2
Adenoma and/or hyperplasia	12	16	17

[a] Data presented here are from Mann and Frame (2004) and only includes rats on study beyond the interim sacrifice at 53 weeks. None of the differences between APFO-treated groups and controls in hyperplasia, adenoma, and combined hyperplasia/adenoma reached statistical significance (Cochran–Armitage trend test and Fisher's exact test, both with significance at $p \leq 0.05$). All of the proliferative lesions diagnosed were gonadal stromal and were based on more recently published nomenclature and corresponded to the diagnosis of tubular hyperplasia or tubular adenoma by the study pathologist (see Table 6). The more generic designation of gonadal stromal for the lesions better reflected the spectrum of morphologic changes observed in the proliferative lesions. Moreover, based on current diagnostic nomenclature, tubular hyperplasia or adenoma would suggest an origin from ovarian surface epithelium rather than from ovarian stromal cells. Size of the proliferative lesion is the primary criterion differentiator between the diagnosis of hyperplasia versus adenoma. Therefore, the assessment included an evaluation of the total incidence of combined hyperplasia and adenoma.

APFO-fed female groups was observed at terminal sacrifice. The interpretation of these changes, in the absence of any observable progressive pathological lesion after 2 years of treatment, was considered as equivocally related to treatment. A subsequent pathology peer review of ovarian tissues (Mann and Frame, 2004) using current pathological criteria found no statistically significant increases in ovarian hyperplasia, adenoma, or adenoma and hyperplasia combined in treated groups relative to controls. While not statistically significant, the controls experienced a somewhat higher incidence of adenoma. Thus, the incidence of ovarian stromal hyperplasia and/or adenoma was not increased by APFO.

Both, the study reported here and the Biegel et al. study, have identified the liver as the principal target organ for non-neoplastic effects and the testes (Leydig cells) as the principal organ for neoplastic effects in male Sprague–Dawley rats given 300 ppm APFO in diet for up to 2 years. It is not clear why there were increases in male hepatocellular and pancreatic acinar cell tumors only in the Biegel et al. study. In the liver, this may be due to application of differing histopathological diagnostic criteria. As noted above for the acinar pancreas, some evidence of acinar cell hyperplasia was evident on peer-review using current criteria. Although sample source and purity and average daily APFO consumption by 300 ppm group males in both studies was quite similar (14.2 mg/kg in this study and 13.6 mg/kg in the Biegel et al. study), the studies used different base diets (Certified Purina Laboratory Chow (Ralston Purina, Inc., St. Louis, Missouri) in this study and Certified Rodent Diet #5002 (PMI Feeds, Inc.) in the Biegel et al. study). Differences in diet composition may have affected bioavailability. Also, dietary factors such as raw soy, diets deficient in choline, and corn oil can affect pancreatic acinar cell tumor outcome in rats (Eustis and Boorman, 1985; Longnecker and Millar, 1990; McGuinness et al., 1980). However, it is not known if differences in base diets were responsible for the differences in outcomes observed between the two studies. Another possibility is temporal differences between the 1980s and 1990s in the Sprague–Dawley rat stock used in these two studies. Both studies used CD Sprague–Dawley stock from Charles River Laboratories; although, rats were sourced from different breeding facilities at times several years apart. The years between studies could have affected the genetic characteristics

Table 8
Incidence of neoplastic microscopic findings for male and female rats in either control groups or groups fed 30 ppm or 300 ppm APFO in their diet for 2 years.

Organ/lesion	Dietary dose group (ppm APFO)					
	Males			Female		
	0	30	300	0	30	300
Adrenal						
Pheochromocytoma, benign	2/49 (4)[a]	4/50 (8)	4/50 (8)	2/50 (4)	0/50 (0)	0/49 (0)
Pheochromocytoma, malignant	0/49 (0)	1/50 (2)	0/50 (2)	0/50 (0)	0/50 (0)	1/49 (2)
Liver						
Hepatocellular adenoma	0/49 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
Hepatocellular carcinoma	3/49 (6)	1/50 (2)	5/50 (10)	0/50 (0)	0/50 (0)	1/50 (2)
Mammary gland						
Adenocarcinoma	–	–	–	7/46 (15)	14/45 (31)	5/44 (11)
Adenoma	–	–	–	3/46 (7)	0/45 (0)	0/44 (0)
Carcinoma	–	–	–	1/46 (2)	0/45 (0)	0/44 (0)
Fibroadenoma	–	–	–	10/46 (22)	19/45 (42)	**21/44 (48)***
Lymphangiosarcoma	–	–	–	0/46 (0)	0/45 (0)	1/44 (2)
Reevaluation by PWG[b]						
Adenocarcinoma				9/50 (18)	16/50 (32)[c]	5/50 (10)[c]
Adenoma				1/50 (2)	0/50 (0)	0/50 (0)
Fibroadenoma				16/50 (32)	16/50 (32)	20/50 (40)
Fibroadenoma (multiple)				2/50 (4)	6/50 (12)	3/50 (6)
Pituitary						
Adenoma	17/48 (35)	17/47 (36)	13/46 (28)	33/46 (72)	39/47 (83)	36/50 (72)
Testes/epididymis						
Leydig cell adenoma	0/49 (0)	2/50 (4)	**7/50 (14)***	–	–	–
Thyroid						
C-cell adenoma	0/43 (0)	2/47 (4)	4/47 (9)	1/50 (2)	0/45 (0)	0/41 (0)
C-cell carcinoma	2/43 (5)	0/47 (0)	0/47 (0)	0/50 (0)	0/45 (0)	0/41 (0)

Bolded values are statistically significant.
* Statistically significantly different from controls ($p \leq 0.05$).
[a] Number observed/number examined (%).
[b] Hardisty et al. (2010).
[c] The incidence in the groups sharing this footnote were statistically significantly different from each other ($p < 0.01$, Hardisty et al., 2010).

of the Sprague–Dawley stock in a manner that could modify response. Indeed, Charles River reported that a trend in decreased longevity of CD Sprague– Dawley rats was noted in the late 1980s, perhaps due to a loss of heterozygosity due to unconscious selection pressures associated with a random mating program (https://www.crj.co.jp/../pdf/rm_rm_a_igs_rat_breeding_system.pdf). This led to a comprehensive outbreeding program and harmonization of worldwide Charles River breeding stocks in 1992. The study here reported was conducted in the mid-1980s; whereas, the study by Biegel et al. was conducted in the early 1990s, but started prior to 1992. Thus, it may be that genetic differences in Sprague–Dawley CD stock over time may have influenced outcomes. However, the basis for the differences in degree of male rat response to APFO treatment between the two studies cannot be determined with any certainty. There may be other potential explanations for differences in outcomes between the two studies, but no conclusion can be reached at present.

In considering the mode of action for the Leydig cell tumor response, the genotoxic potential of APFO (PFOA) has been studied, and APFO is not considered to be genotoxic agent (Johansson et al., 2009; Kennedy et al., 2004; Lau et al., 2007). Therefore, the increased incidence of Leydig cell adenoma likely occurs through a non-genotoxic mode of action.

There are three epidemiologic studies that have investigated exposure to PFOA and cancer-related outcomes, two involving cancer mortality in occupational cohorts (Leonard et al., 2008; Lundin et al., 2009) and the third involving the Danish general population. The occupational cohorts had workers with average serum PFOA concentrations in the 500–1000 ng/mL range; whereas, the Danish general population's serum concentration was two orders of magnitude lower. The first occupational cohort included 3M workers that manufactured APFO. In the most recent study update of the 3M cohort ($n = 3997$ workers), Lundin et al. (2009) reported deaths from three liver cancers (SMR 0.5, 95% CI), 13 pancreatic cancers (SMR 0.9, 95% CI 0.5–1.5), and 0 testicular cancers. Although Lundin et al. concluded liver and pancreatic cancers were not associated with PFOA in their study, they did observe an association with prostate cancer; however, Lundin et al. caution that this analysis was primarily the consequence of a statistically significant deficit of prostate cancer in the least exposed workers (SMR 0.4, 95% CI 0.1–0.9). In the second occupational cohort, Leonard et al. (2008) reported the mortality experience of 6027 DuPont workers employed at a facility at which approximately 30% of the workers were assigned to work areas in which APFO was used as a processing aid in the polymerization of fluoropolymers, and the remaining approximately 70% had possible indirect exposure to APFO. Altogether, there were eight deaths from liver cancer compared to 5.5 expected (1.5 (95% CI 0.6–2.9), 11 deaths from pancreatic cancer compared to 11.2 expected (SMR 1.0, 95% CI 0.5–1.8), and 12 deaths from prostate cancer compared to 17.1 expected (SMR 0.7, 95% CI 0.3–1.1) based on the company's reference population. Leonard et al. did observe an association with kidney cancer, with 12 deaths compared to 6.6 expected (SMR 1.8, 95% CI 0.9–3.2). The third study was a case–cohort analysis of a Danish general population that examined baseline serum PFOA concentrations related to the incidence of liver, pancreatic, prostate, and bladder cancer (Eriksen et al., 2009). Eriksen et al. followed a cohort of 57,033 individuals aged 50–65 over a 13-year time period and reported 66 liver, 128 pancreatic, 713 prostate, and 332 bladder cancers. Baseline serum PFOA concentrations of these cancer cases were compared to 772 controls whose mean PFOA level was 6.6 ng/mL (range 1–27 ng/mL). Incidence rate ratios (IRR) were calculated by each quartile of serum PFOA observed in the control group. When compared to the 1st quartile (IRR = 1.0) the IRR values for the 2nd, 3d, and 4th quartiles were: for liver cancer, 0.5, 0.6, and 1.0; for pancreatic cancer, 0.9, 1.3, and 1.6; for prostate cancer, 1.1, 0.9, and 1.2; and for bladder cancer, 0.9, 0.8, and 1.0. No statistically significant trends were observed, and it was concluded that the incidences of these four cancers were not associated with serum PFOA in the Danish general population.

In summary, a 2-year dietary study in rats fed up to 300 ppm APFO found the liver to be the primary target as evidenced by changes in clinical enzymes and tissue histopathology. An increase in the incidence of testicular Leydig cell adenomas was observed in males fed 300 ppm APFO but not in males fed 30 ppm APFO. The types and incidences of all other tumors reflect those expected to be observed spontaneously in Sprague–Dawley rats and were not associated with dietary exposure to APFO.

Conflict of interest

John L. Butenhoff, Shu-Ching Chang and Geary W. Olsen are employees of 3M Company, a former manufacturer of ammonium PFOA and the company supporting the work reported on in the article. Gerald L. Kennedy, Jr. represents DuPont Company, a current manufacturer and user of ammonium PFOA.

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